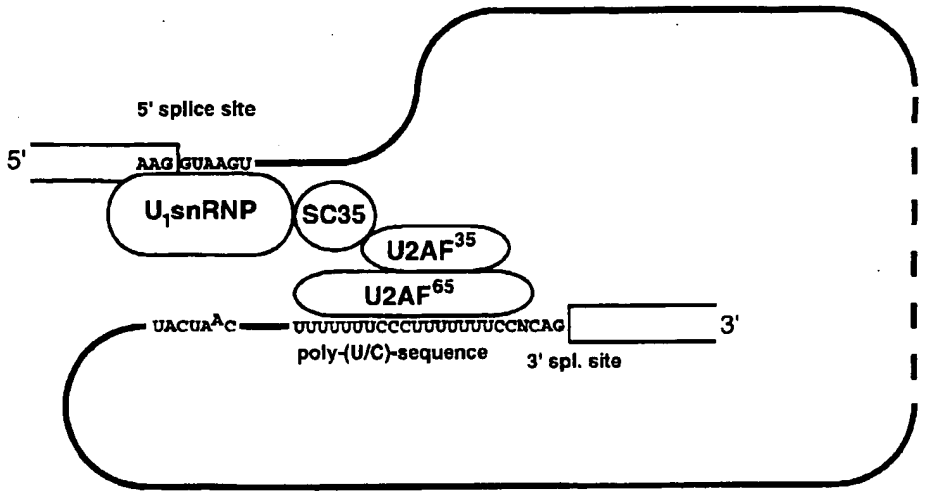
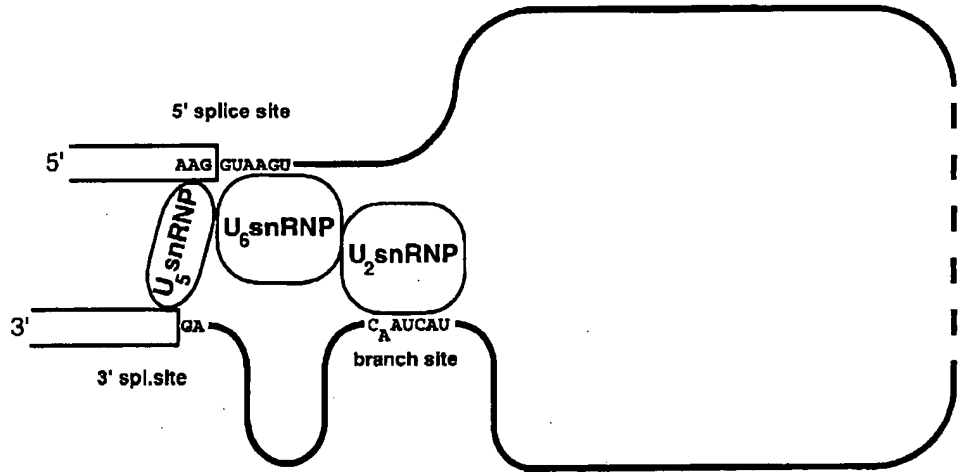
A

(1)

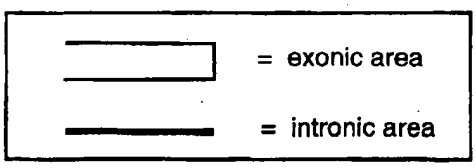


association between the 5' splice site and the 3' splice site in the E-complex

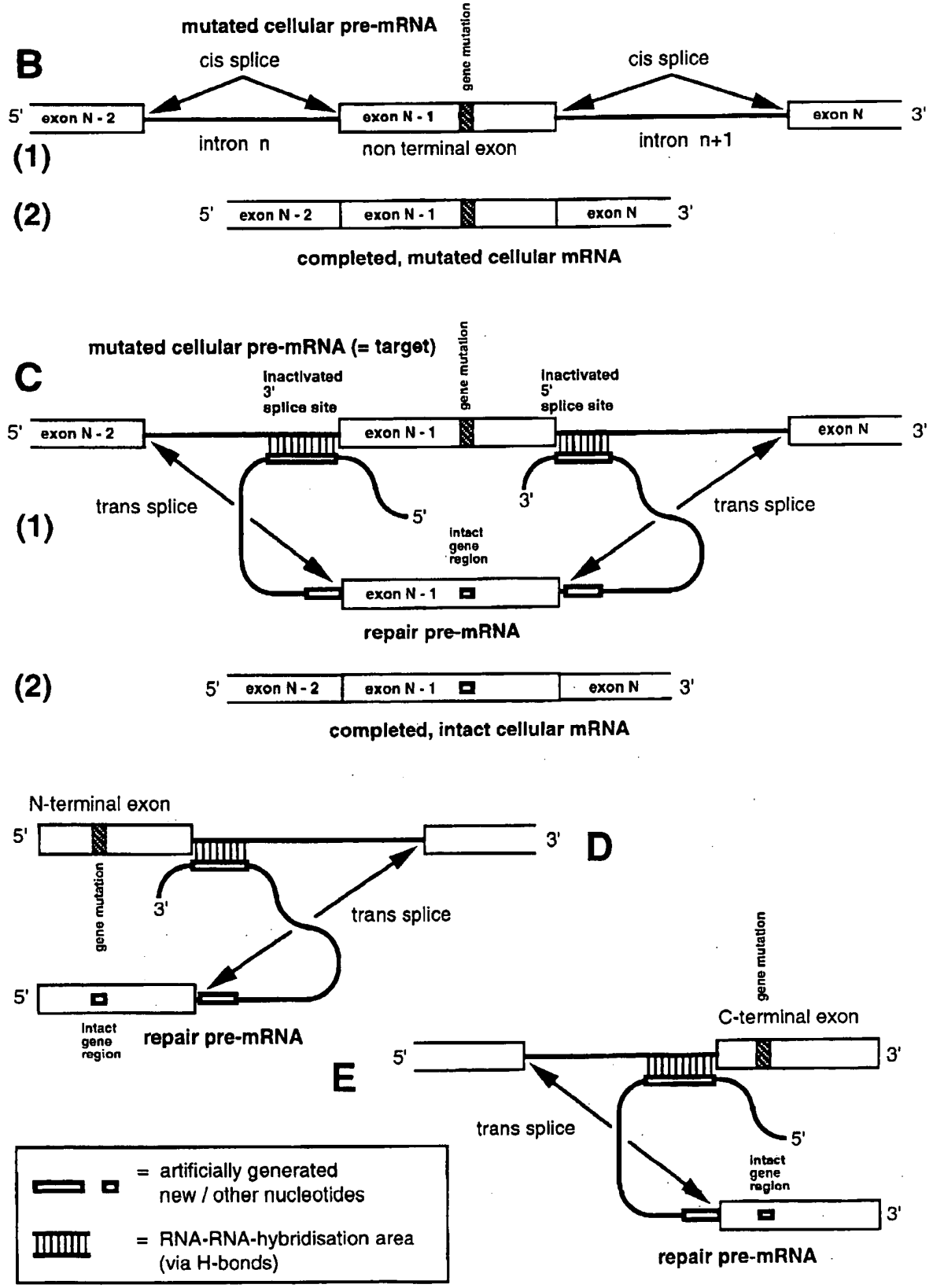
(2)



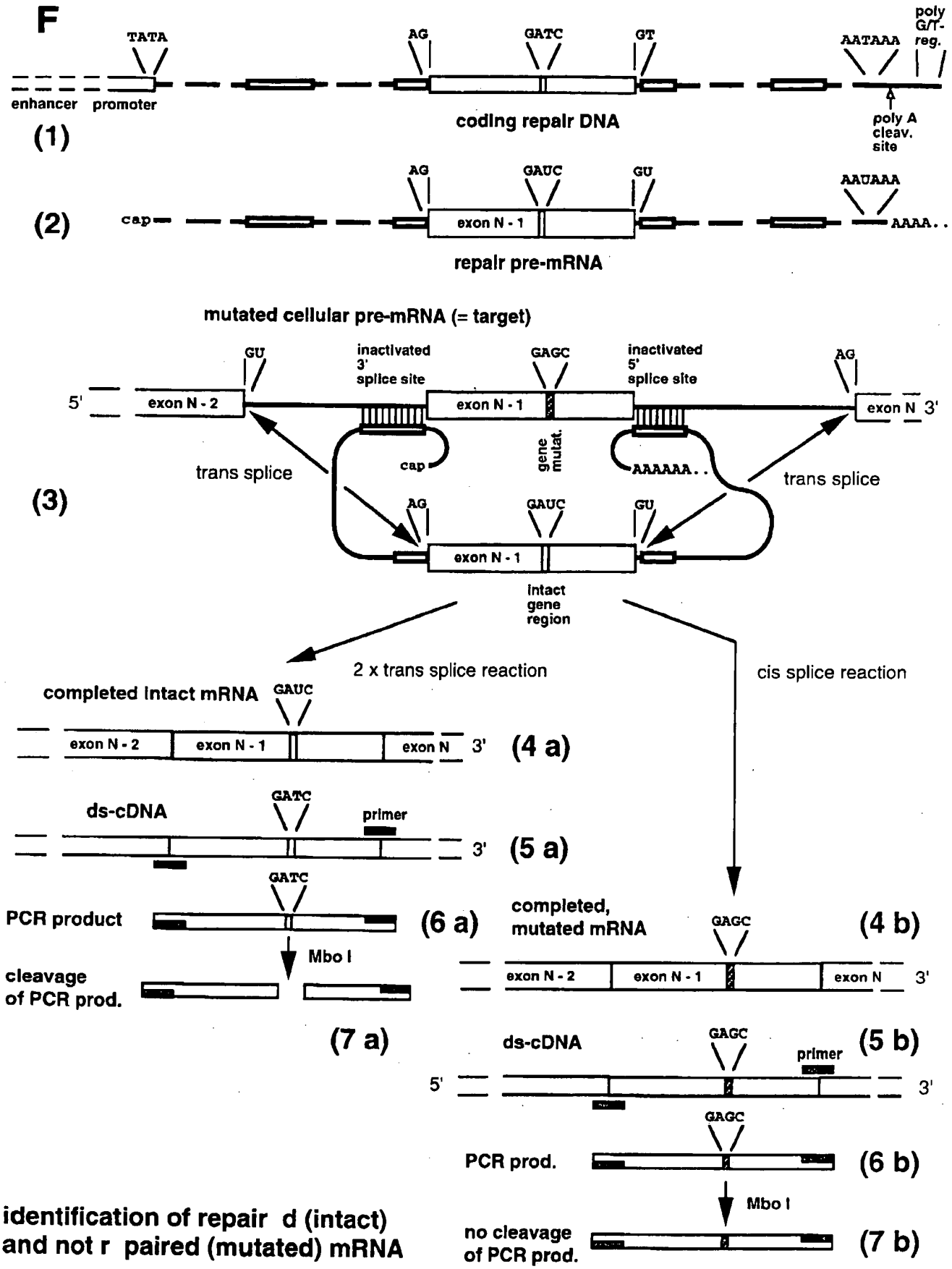
association between the 5' splice site and the 3' splice site in the B/C-complex

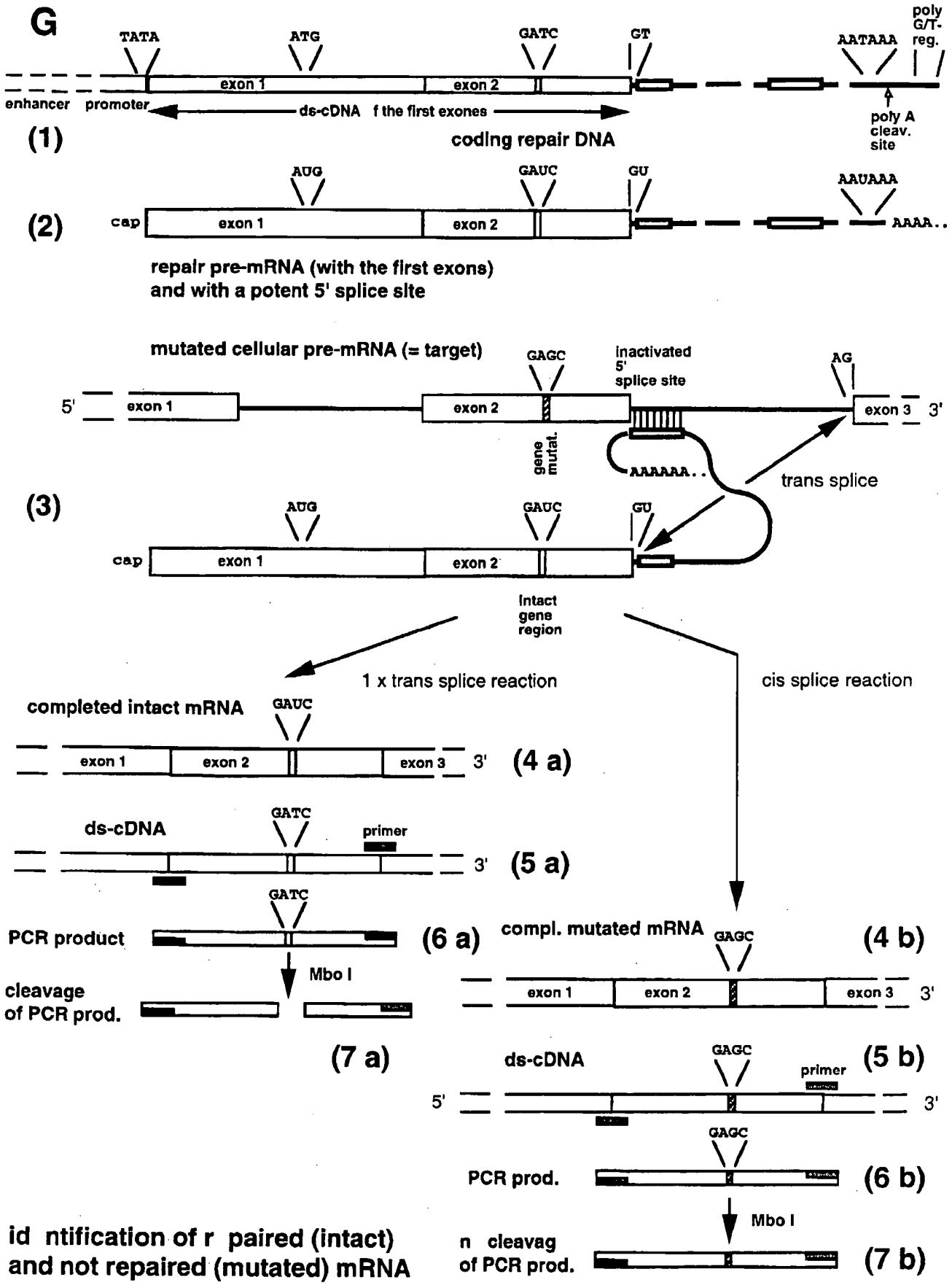


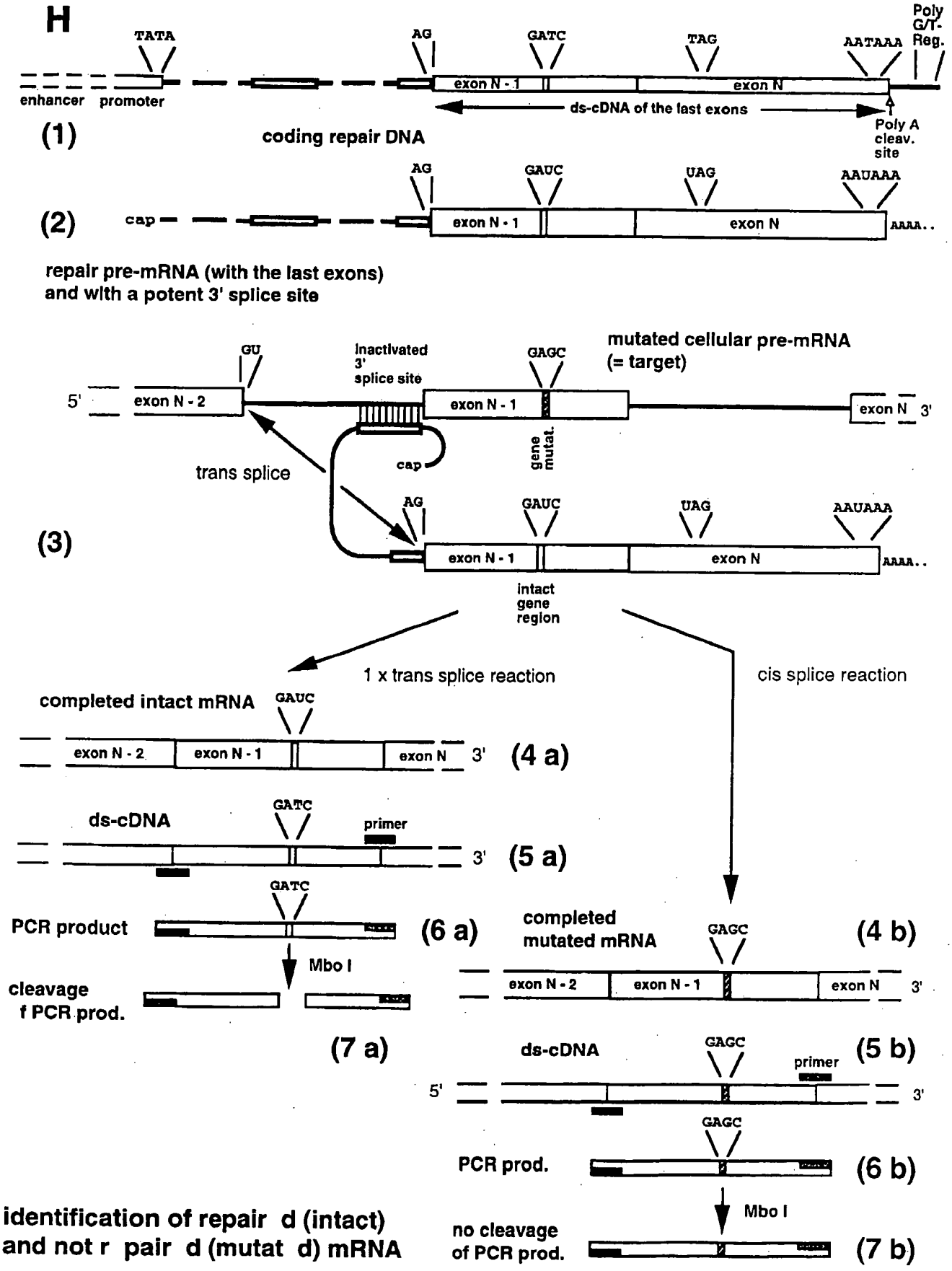
association b tween the 5' and 3' splic site at different points during splicing

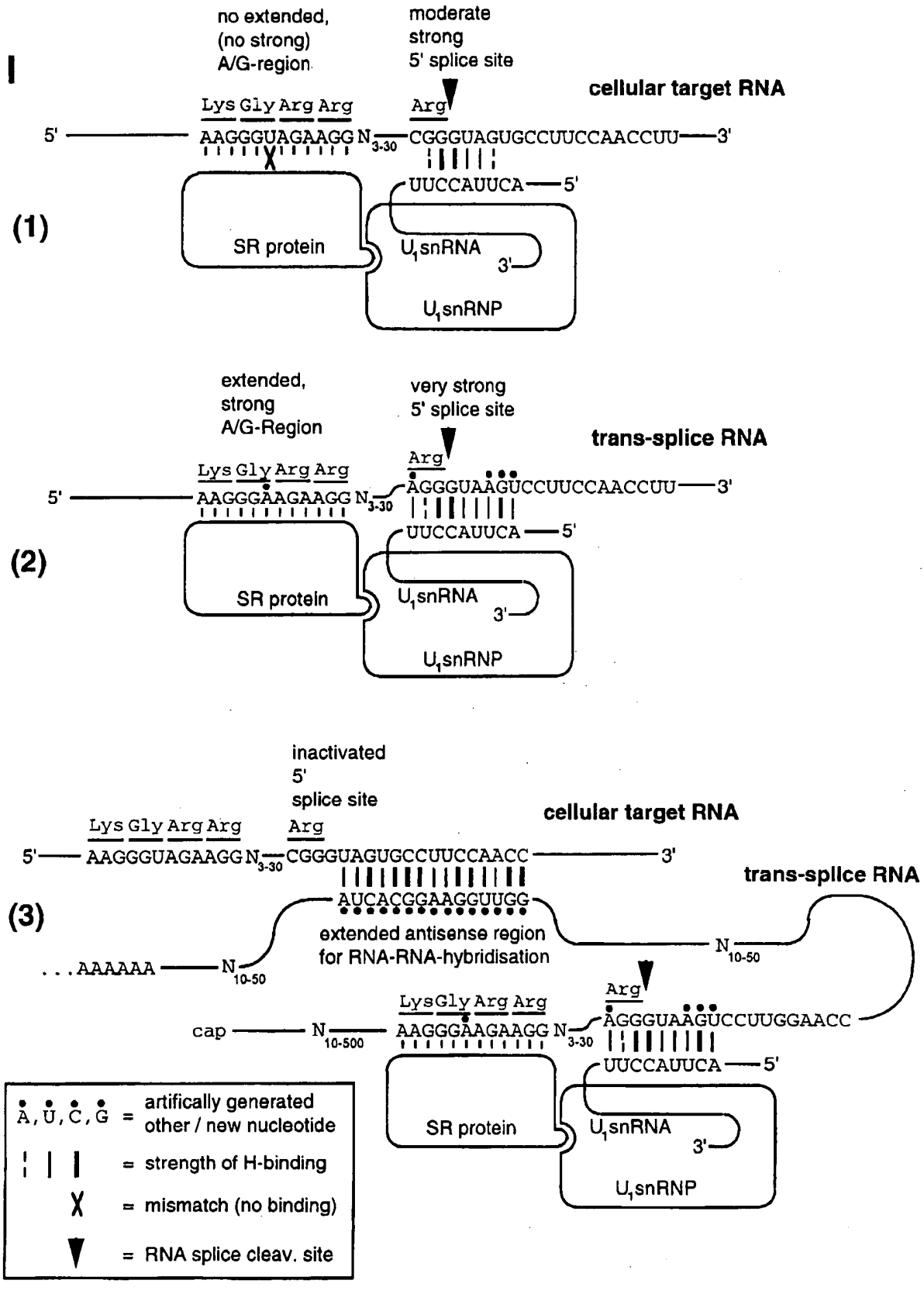


repair of int rnal, N- or C-terminal gene mutated exons by trans splicing

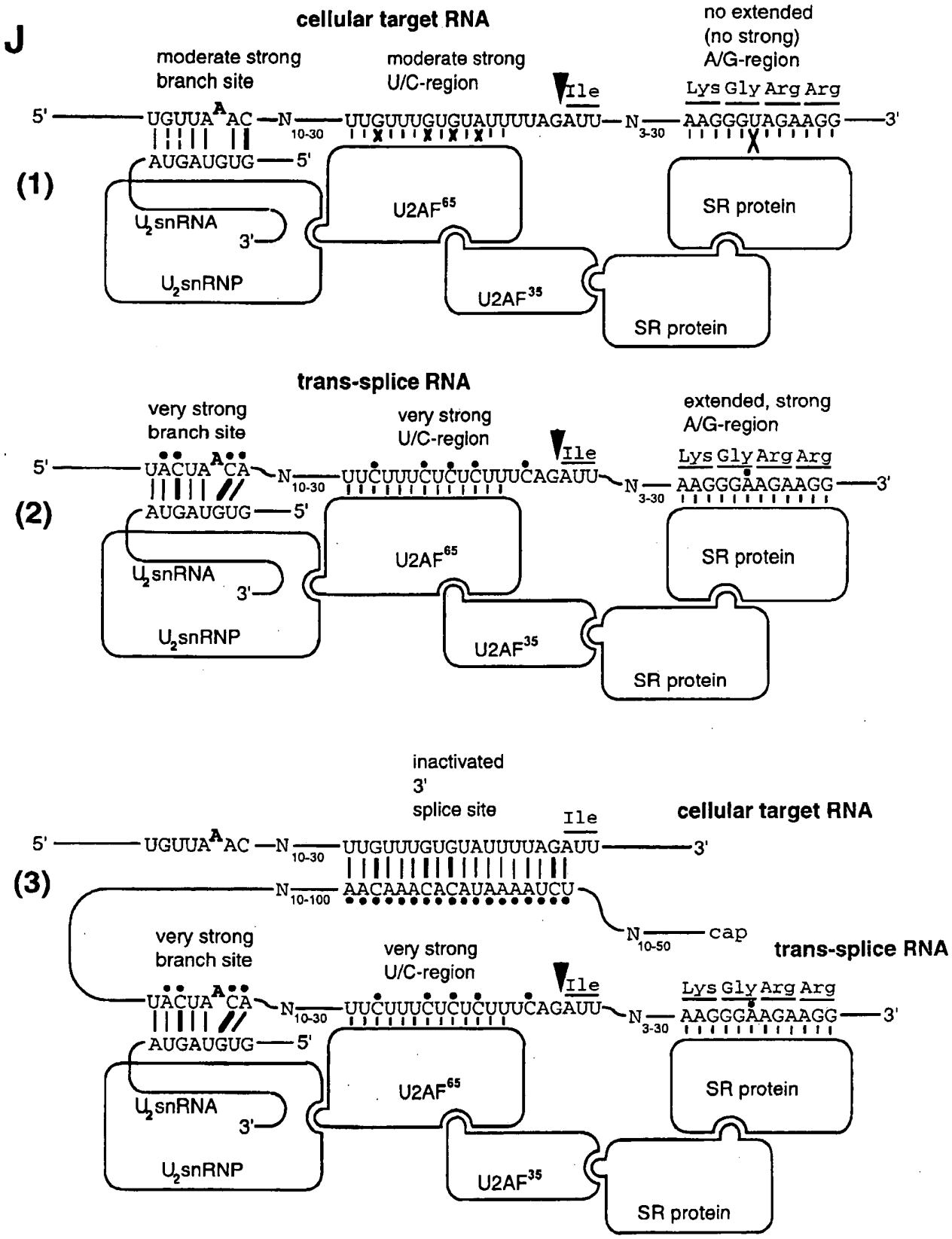

F
TATA
AG
GATC
GT
AATAAA
poly G/T-reg.
enhancer
promoter
coding repair DNA
poly A cleav. site
(1)
AG
GAUC
GU
AAUAAA
(2)
cap
exon N - 1
AAAA..
repair pre-mRNA
mutated cellular pre-mRNA (= target)
GU
inactivated 3' splice site
GAGC
inactivated 5' splice site
AG
5'
exon N - 2
exon N - 1
exon N
3'
trans splice
cap
gene mutat.
AAAAAA..
trans splice
(3)
AG
GAUC
GU
exon N - 1
intact gene region
2 x trans splice reaction
cis splice reaction
completed intact mRNA
GAUC
exon N - 2
exon N - 1
exon N
3'
(4 a)
ds-cDNA
GATC
primer
3'
(5 a)
GATC
PCR product
(6 a)
Mbo I
cleavage of PCR prod.
(7 a)
completed, mutated mRNA
GAGC
(4 b)
exon N - 2
exon N - 1
exon N
3'
ds-cDNA
GAGC
primer
(5 b)
5'
3'
GAGC
PCR prod.
(6 b)
Mbo I
no cleavage of PCR prod.
(7 b)
identification of repair d (intact) and not r paired (mutated) mRNA


G
TATA
ATG
GATC
GT
AATAAA
poly G/T- reg.
enhancer
promoter
exon 1
exon 2
ds-cDNA f the first exones
poly A cleav. site
(1)
coding repair DNA
AUG
GAUC
GU
AAUAAA
(2)
cap
exon 1
exon 2
AAAA..
repair pre-mRNA (with the first exons) and with a potent 5' splice site
mutated cellular pre-mRNA (= target)
GAGC
inactivated 5' splice site
AG
5'
exon 1
exon 2
exon 3
3'
gene mutat.
AAAAAA..
trans splice
(3)
AUG
GAUC
GU
cap
exon 1
exon 2
Intact gene region
1 x trans splice reaction
cis splice reaction
completed intact mRNA
GAUC
exon 1
exon 2
exon 3
3'
(4 a)
ds-cDNA
GATC
primer
3'
(5 a)
GATC
PCR product
(6 a)
Mbo I
cleavage of PCR prod.
(7 a)
compl. mutated mRNA
GAGC
(4 b)
exon 1
exon 2
exon 3
3'
ds-cDNA
GAGC
primer
(5 b)
5'
3'
GAGC
PCR prod.
(6 b)
Mbo I
n cleavag of PCR prod.
(7 b)
id ntification of r paired (intact) and not repaired (mutated) mRNA

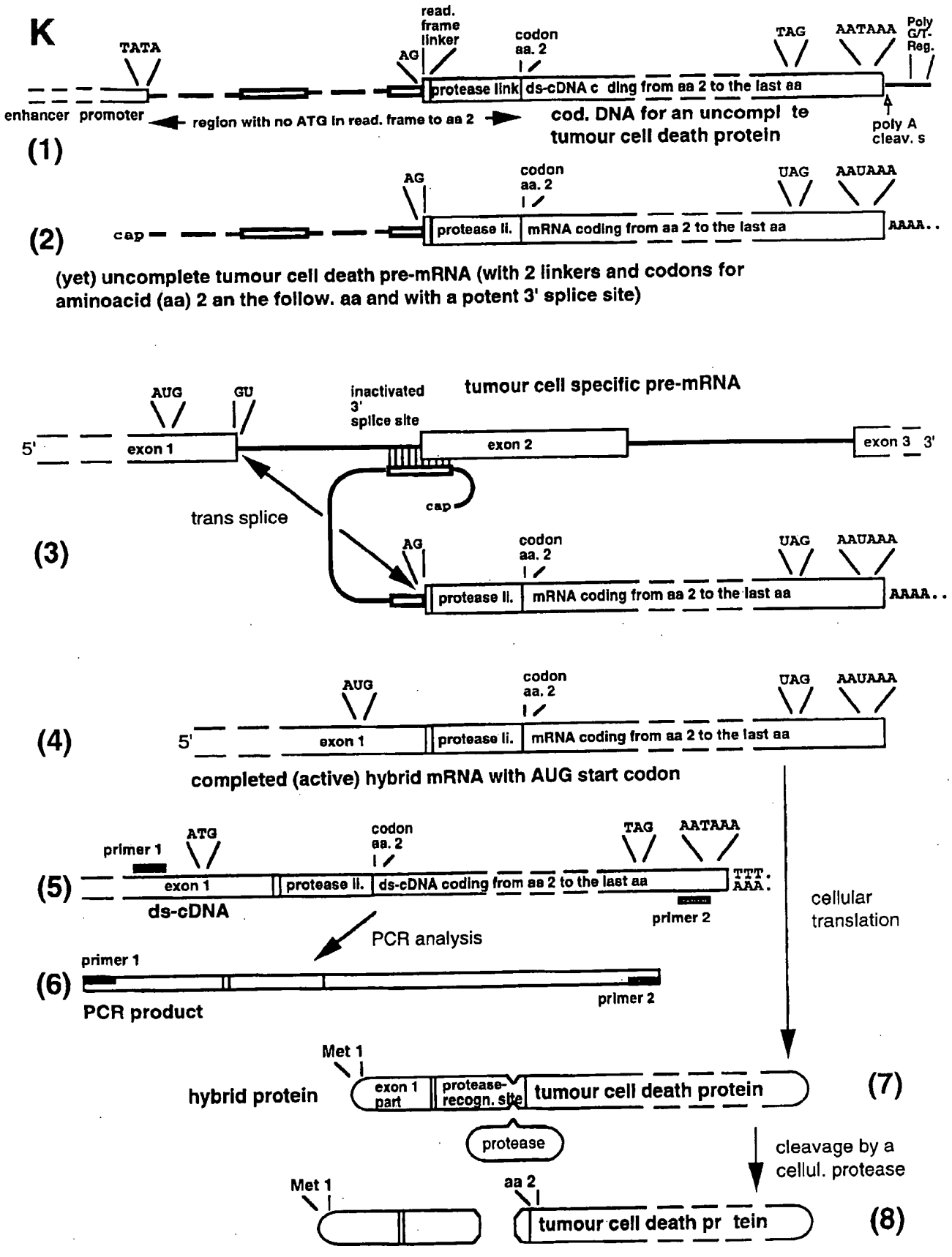

H
TATA
AG
GATC
TAG
AATAAA
Poly G/T-Reg.
enhancer
promoter
exon N - 1
exon N
ds-cDNA of the last exons
Poly A cleav. site
(1)
coding repair DNA
AG
GAUC
UAG
AAUAAA
(2)
cap
exon N - 1
exon N
AAAA..
repair pre-mRNA (with the last exons)
and with a potent 3' splice site
GU
inactivated 3' splice site
GAGC
mutated cellular pre-mRNA
(= target)
5'
exon N - 2
exon N - 1
exon N
3'
trans splice
cap
gene mutat.
(3)
intact gene region
1 x trans splice reaction
cis splice reaction
completed intact mRNA
(4 a)
ds-cDNA
primer
(5 a)
PCR product
(6 a)
Mbo I
cleavage f PCR prod.
(7 a)
completed mutated mRNA
(4 b)
ds-cDNA
(5 b)
PCR prod.
(6 b)
no cleavage of PCR prod.
(7 b)
identification of repair d (intact)
and not r pair d (mutat d) mRNA



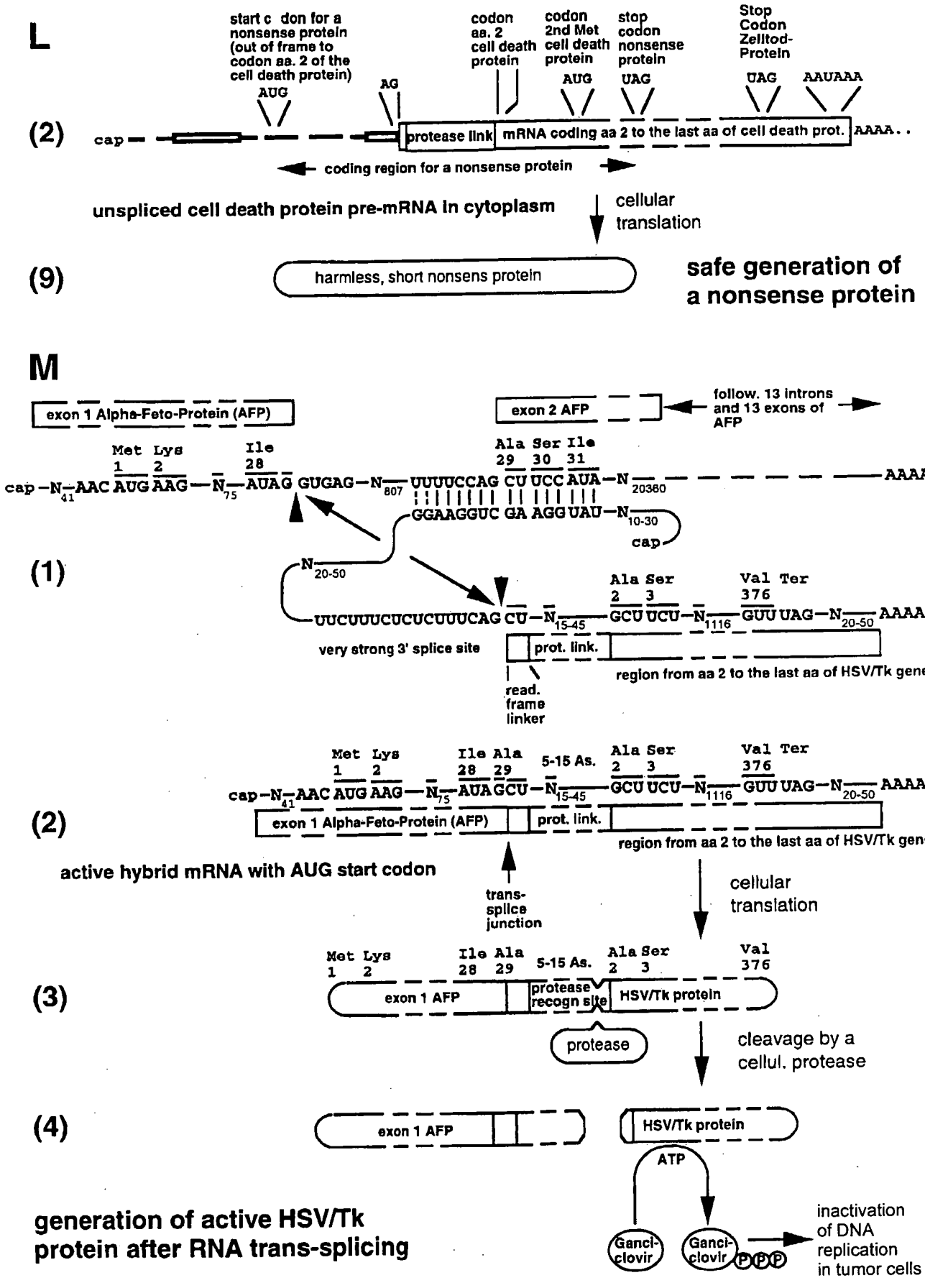
methodes to pr f r the us of a 5' splice site on a trans-splice RNA

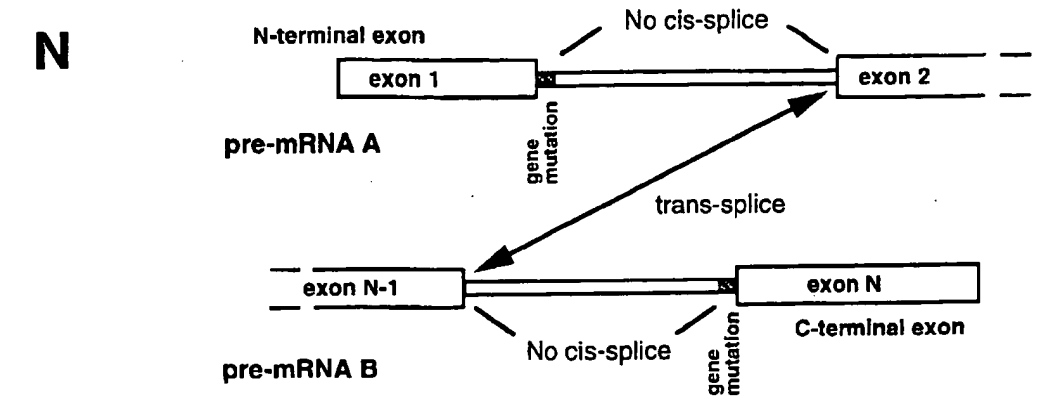


methodes to prefer the use of a 3' splice sit on a trans-splic RNA

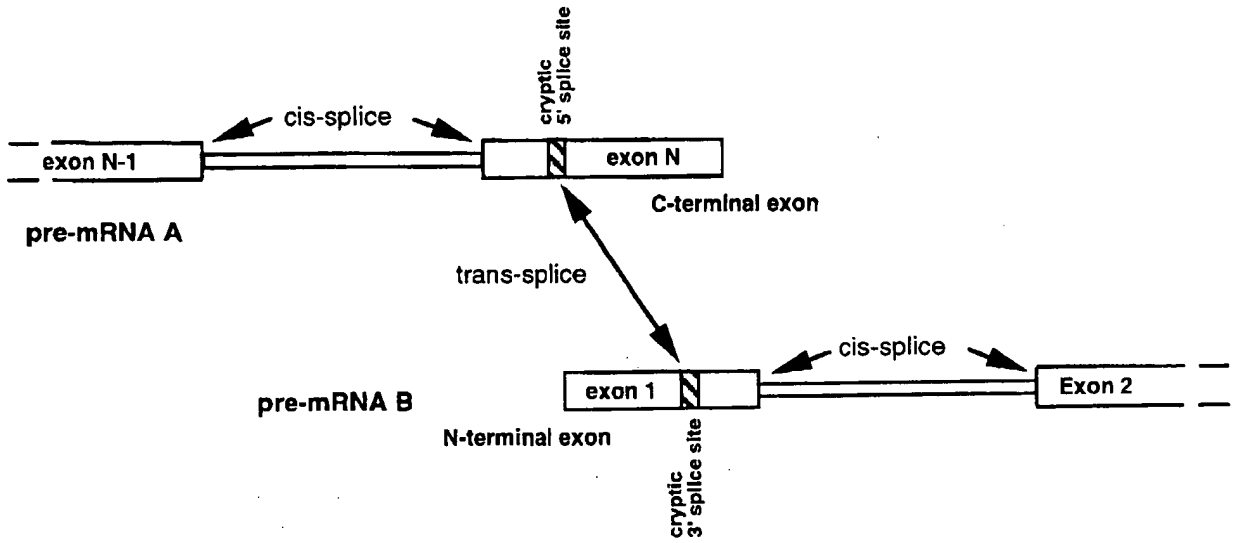


gen rati n of deadly proteins in tumour c lls after trans splicing

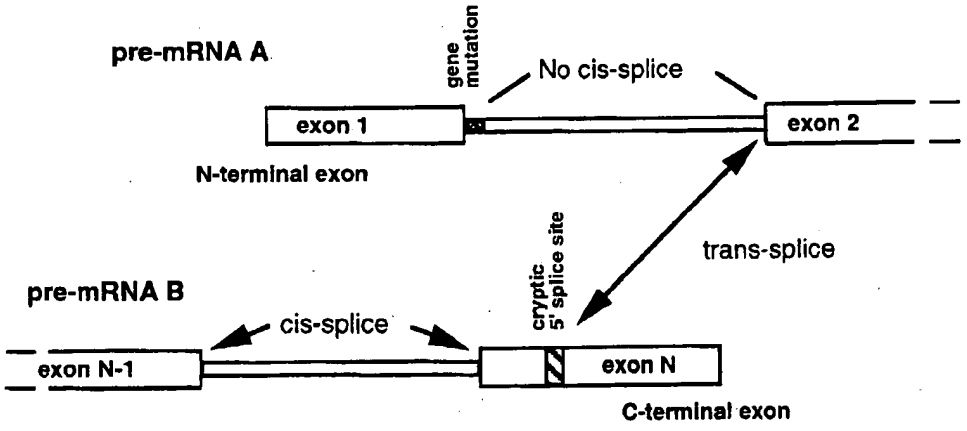

L
start c don for a nonsense protein (out of frame to codon aa. 2 of the cell death protein)
AUG
AG
codon aa. 2 cell death protein
codon 2nd Met cell death protein
AUG
stop codon nonsense protein
UAG
Stop Codon Zelltod-Protein
UAG
AAUAAA
(2)
cap
protease link
mRNA coding aa 2 to the last aa of cell death prot.
AAAA..
coding region for a nonsense protein
unspliced cell death protein pre-mRNA in cytoplasm
cellular translation
(9)
harmless, short nonsens protein
safe generation of a nonsense protein
M
exon 1 Alpha-Feto-Protein (AFP)
exon 2 AFP
follow. 13 introns and 13 exons of AFP
Met Lys Ile
1 2 28
Ala Ser Ile
29 30 31
cap–N41–AAC AUG AAG–N75–AUAG GUGAG–N807–UUUUCCAG CU UCC AUA–N20360–AAAA..
GGAAGGUC GA AGG UAU–N10-30
cap
(1)
N20-50
Ala Ser
2 3
Val Ter
376
UUCUUUCUCUCUUUCAG CU–N15-45–GCU UCU–N1116–GUU UAG–N20-50–AAAA..
very strong 3' splice site
prot. link.
region from aa 2 to the last aa of HSV/Tk gene
read. frame linker
Met Lys
1 2
Ile Ala
28 29
5-15 As.
Ala Ser
2 3
Val Ter
376
cap–N41–AAC AUG AAG–N75–AUA GCU–N15-45–GCU UCU–N1116–GUU UAG–N20-50–AAAA..
(2)
exon 1 Alpha-Feto-Protein (AFP)
prot. link.
region from aa 2 to the last aa of HSV/Tk gene
active hybrid mRNA with AUG start codon
trans-splice junction
cellular translation
Met Lys
1 2
Ile Ala
28 29
5-15 As.
Ala Ser
2 3
Val
376
(3)
exon 1 AFP
protease recogn site
HSV/Tk protein
protease
cleavage by a cellul. protease
(4)
exon 1 AFP
HSV/Tk protein
ATP
Ganci-clovir
Ganci-clovir
P P P
inactivation of DNA replication in tumor cells
generation of active HSV/Tk protein after RNA trans-splicing



(1) stimulation of trans-splicing by mutations in the cis splice sites in N- or C-terminal introns



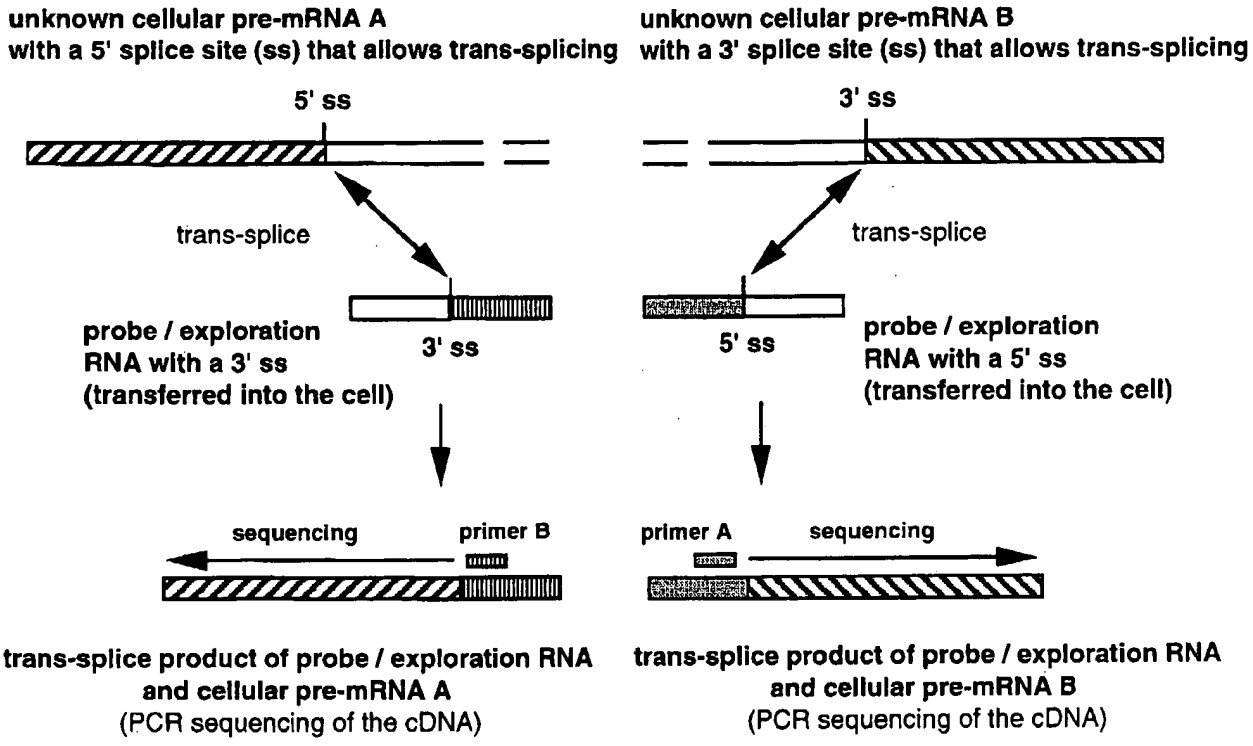
(2) stimulation of trans-splicing by activating cryptic splice sites in N- or C-terminal exons



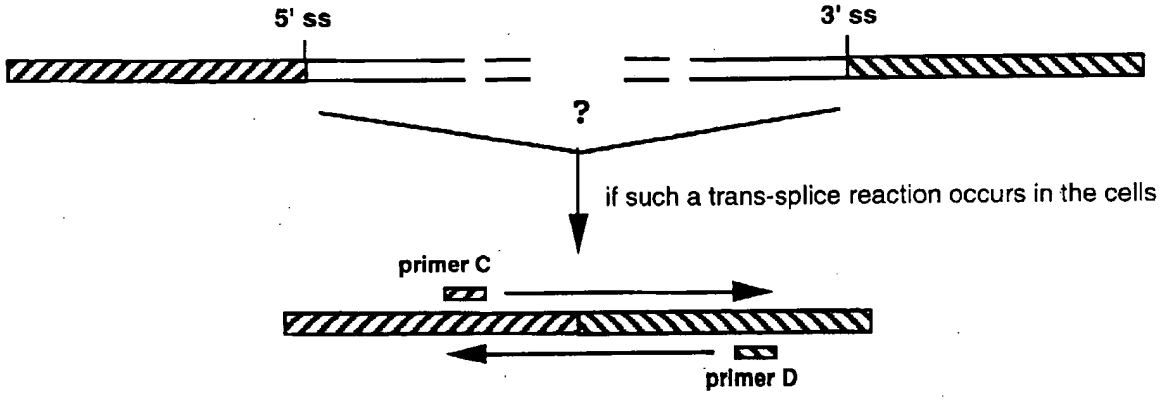
(3) stimulation of trans-splicing by activating a cryptic splice site in N- or C-terminal exon and a mutation in a cis-splice site in a N-terminal intron

O

(1) step 1: idendification potential pre-mRNAs for RNA trans-splicing



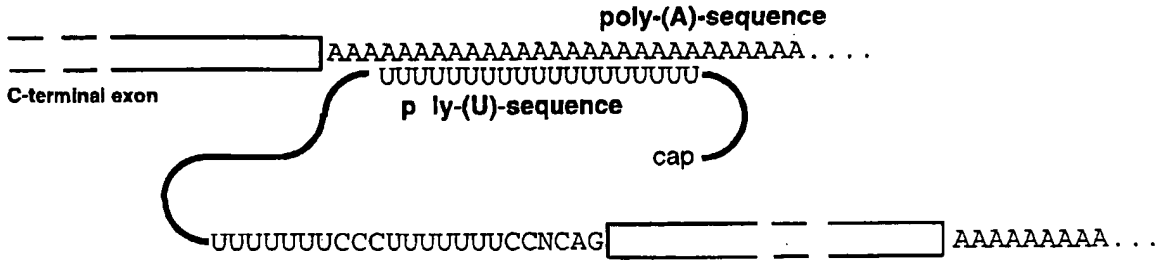
(2) step 2: idendification of potential natural cellular trans-splice products



principl of identification of yet unknown c llular mRNA trans-splice products

P

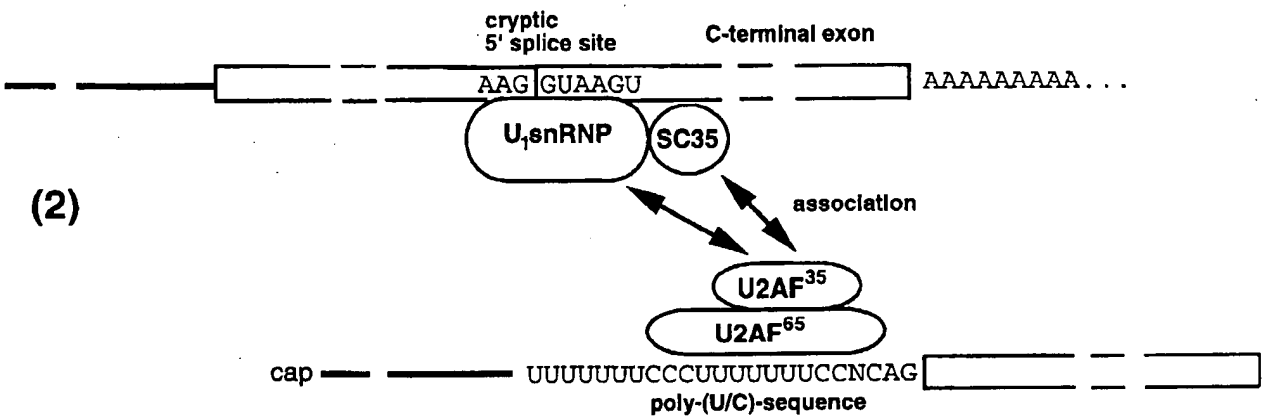
unkn wn cellular pr -mRNA
with a 5' splice sit (ss) that allows trans-splicing



probe / exploration RNA with a 3' ss
(transferred into the cell)

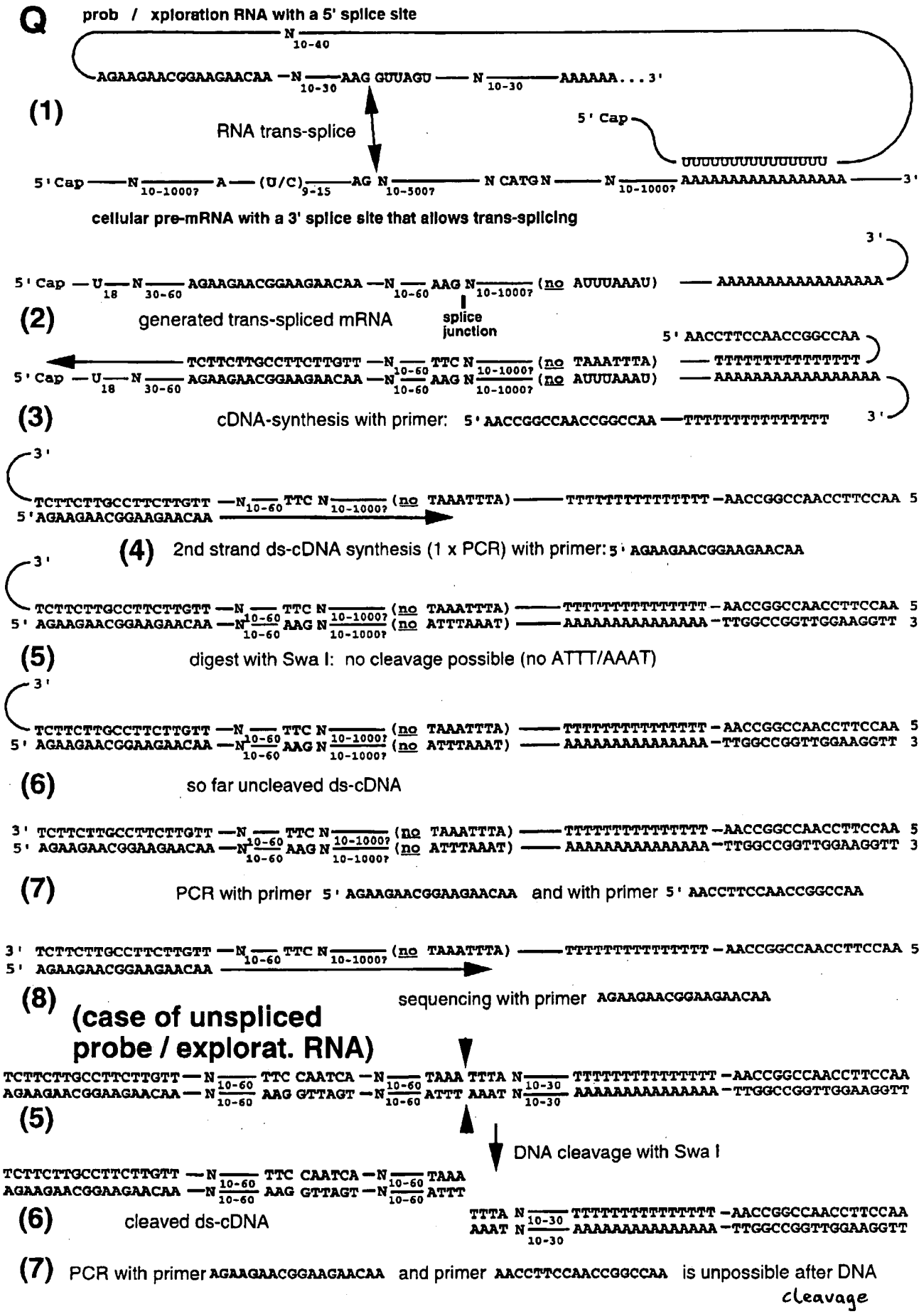
association between a probe / exploration RNA and a cellular pre-mRNA that allows trans-spling by antisense pairing between a poly-U-region on the probe / exploration RNA and the poly-A-tail-region on the cellular pre-mRNA

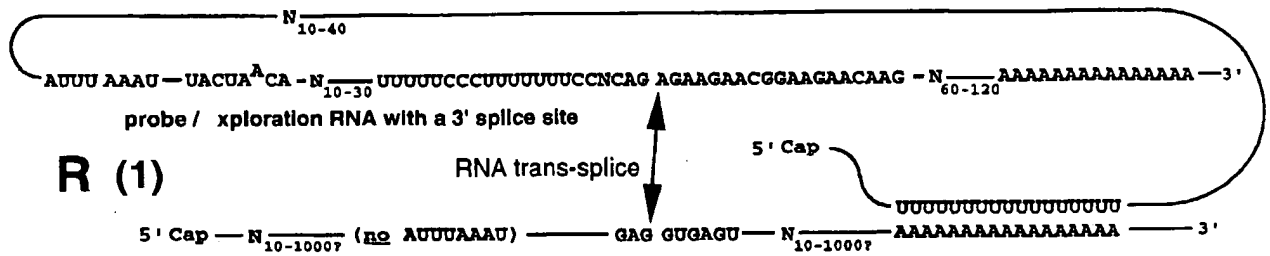
unknown pre-mRNA
with a (crypric) splice site that allows trans-splicing and bound U_1snRNP



probe / exploration RNA with a 3' ss and with bound U2AF complex

association of the 3' splice site on a probe / exploration RNA
to a cryptic 5' splice site in a cellular pre-mRNA that allows trans-splicing
by previously bound splice proteins in the E-complex


Q
prob / xploration RNA with a 5' splice site
AGAAGAACGGAAGAACAA
AAG GUUAGU
AAAAAA...3'
(1)
RNA trans-splice
5' Cap
UUUUUUUUUUUUUUU
5' Cap
A
(U/C)
AG
N CATG N
AAAAAAAAAAAAAAAAA
3'
cellular pre-mRNA with a 3' splice site that allows trans-splicing
5' Cap
AGAAGAACGGAAGAACAA
AAG
(no AUUUAAAU)
AAAAAAAAAAAAAAAAA
(2)
generated trans-spliced mRNA
splice junction
5' AACCTTCCAACCGGCCAA
TCTTCTTGCCTTCTTGTT
TTC
(no TAAATTTA)
TTTTTTTTTTTTTTT
(3)
cDNA-synthesis with primer: 5' AACCGGCCAACCGGCCAA
(4)
2nd strand ds-cDNA synthesis (1 x PCR) with primer: 5' AGAAGAACGGAAGAACAA
(no ATTTAAAT)
AAAAAAAAAAAAAAA
TTGGCCGGTTGGAAGGTT
(5)
digest with Swa I: no cleavage possible (no ATTT/AAAT)
(6)
so far uncleaved ds-cDNA
(7)
PCR with primer 5' AGAAGAACGGAAGAACAA and with primer 5' AACCTTCCAACCGGCCAA
(8)
sequencing with primer AGAAGAACGGAAGAACAA
(case of unspliced probe / explorat. RNA)
TCTTCTTGCCTTCTTGTT
TTC CAATCA
TAAA TTTA
AACCGGCCAACCTTCCAA
AGAAGAACGGAAGAACAA
AAG GTTAGT
ATTT AAAT
TTGGCCGGTTGGAAGGTT
(5)
DNA cleavage with Swa I
TTTA
AAAT
(6)
cleaved ds-cDNA
(7)
PCR with primer AGAAGAACGGAAGAACAA and primer AACCTTCCAACCGGCCAA is unpossible after DNA cleavage



R (1)

(2) generated trans-spliced mRNA (splice junction)

(3) cDNA-synthesis with primer: TTTTTTTTTTTTTTT and after that terminal elongation with cytosins (C)

(4) (a) annealing of primer: GGTTGGAAGGTTGGAAGGGGGG

(4) (b) complete nucleot. completion to the primer sequence

(4) (c) 2nd strand ds-cDNA synthes. (1 x PCR) with primer: 5' GGTTGGAAGGTTGGAAG

(5) digest with Swa I: no cleavage possible (no ATTT/AAAT)

(6) so far uncleaved ds-cDNA

(7) PCR with primer 5' GGTTGGAAGGTTGGAAG and with primer 5' CTTGTTCTTCCGTTCTTCT

(8) sequencing with primer GGTTGGAAGGTTGGAAG

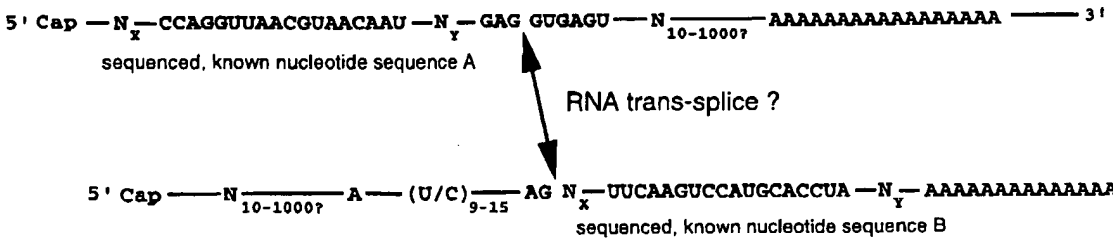
(case of unspliced probe / explorat. RNA)

(5) + (6) DNA cleavage with Swa I

(7) PCR with primer GGTTGGAAGGTTGGAAG and primer CTTGTTCTTCCGTTCTTCT is unpossible after DNA cleavage

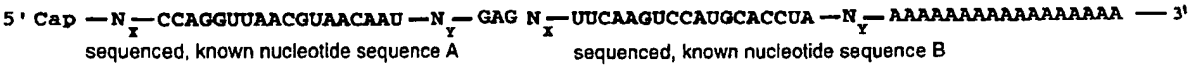
S

cellular pre-mRNA A with a 5' splice sit that allows trans-splicing

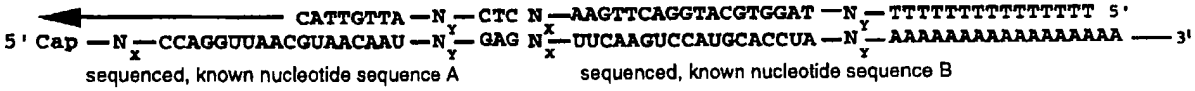


(1)

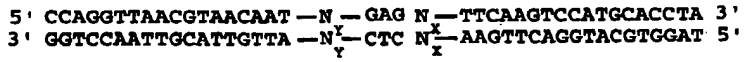
cellular pre-mRNA A with a 3' splice site that allows trans-splicing



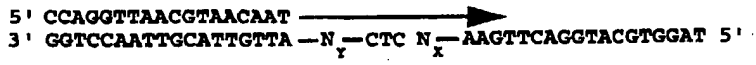
(2) if RNA trans-splice occurs in vivo: generated cellular hybrid mRNA



(3) cDNA synthesis with primer TTTTTTTTTTTTTTT



(4) PCR with primer 5' CCAGGTTAACGTAACAAT and primer 5' TAGGTGCATGGACTTGAA



(5) confirming sequencing with primer CCAGGTTAACGTAACAAT

final evidence on natural cellular trans-splice products
generated by trans-splicing between two pre-mRNAs that both allow trans-splicing